Disinfectant and Antimicrobial Susceptibility Profiles of *Campylobacter coli* Isolated in 1998 to 1999 and 2015 from Swine and Commercial Pork Chops

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Abstract: Susceptibility profiles were determined for 111 *Campylobacter coli* strains obtained in 1998 to 1999 and 2015 from market age pigs and pork chops against 22 disinfectants and 9 antimicrobials. Resistance to tetracycline (TET) was observed in 44.4% of 1998 to 1999 strains, and the antibiotic resistance profile was TET. But strains obtained in 2015 from swine and retail pork chops had 75% TET resistance and the antibiotic resistance profile was TET, followed by azithromycin-erythromycin-TET-telithromycin-clindamycin. Antimicrobial resistance increased in 2015 strains. All strains were resistant to triclosan, and 84.1% and 95.8% of strains in 1998 to 1999 and 2015, respectively, were chlorhexidine resistant. All strains were susceptible to benzalkonium chloride. There was a shift toward higher susceptibility to chlorhexidine, triclosan, P-128, OdoBan, CPB, and CPC in 2015 swine and pork chop strains compared with 1998 to 1999 strains. The disinfectants Tek-Trol and providone-iodine, tris(hydroxylmethyl)nitromethane (THN) and formaldehyde demonstrated the highest susceptibilities. Didecyldimethylammonium chloride (C10AC) appeared to be about equally effective as benzyldimethyltetradecylammonium chloride (C14BAC) for inhibiting *C. coli*, and both were more effective than C8AC and C12BAC, but C16BAC was not efficient at inhibiting *C. coli*. The BACs, C12BAC and C14BAC, were the most effective ingredients in DC&R. Also, C12BAC and C14BAC, or these two in synergy with C10AC were responsible for inhibition of *C. coli* at high P-128 MICs. No cross-resistance was observed between antibiotics and disinfectants. The continued use of THN and formaldehyde in DC&R should be evaluated since these components are not effective, and their inclusion adds unwanted chemicals in the environment.

Keywords: antimicrobial, *Campylobacter coli*, disinfectant, susceptibility, swine

Practical Application: *Campylobacter* species cause diarrheal disease throughout the world. Disinfectants are often used on the farm, in veterinary medicine, by the food processing industry, in restaurants, and in consumer's homes. Limited information is available in the literature showing how disinfectants or disinfectant components may affect the many different foodborne pathogens, and, specifically, *Campylobacter coli* studied here. The knowledge generated in this study concerning the interactions of a broad array of disinfectants against *C. coli* may well affect the types of disinfectants and disinfectant formulations allowable for use by medical personnel, producers, food processors, restaurants, and consumers.

Introduction

Campylobacter coli is one of two main *Campylobacter* spp. often correlated with human foodborne illnesses (Baer, Miller, & Dilger, 2013; Epps et al., 2013; Nachamkin, Szymanski, & Blaser, 2008; Sifré et al., 2015). *Campylobacter* spp. are rod-shaped Gram-negative bacteria (Penner, 1988; WHO, 2018) that cause diarrheal disease throughout the world (CDC, 2011; EFSA, 2016; Gillespie et al., 2002; Kempf et al., 2017; Mukherjee, Ramamurthy, Bhattacharya, Rajendran, & Mukhopadhyay, 2013; WHO, 2018). The top five major foodborne pathogens in the United States include the *Campylobacter* spp., and the Centers for Disease Control and Prevention has estimated that each year *Campylobacter* foodborne infections cause 845,024 illnesses, 8,463 hospitalizations, and 76 deaths in the United States (CDC, 2011; Scallan et al., 2011). *Campylobacter jejuni* is the most often observed cause of campylobacteriosis (EFSA, 2016). But the predominant *Campylobacter* species found in the intestines of pigs and on pork meat products is *C. coli* (APHIS, 2008; Oporto, Esteban, Aduriz, Juste, & Hurtado, 2007), and the percentage of *C. coli* caused campylobacteriosis in some areas of the world may be as high as 35% to 40% (Blackburn & McClure, 2009). The risk factors for transmission of *C. coli* to humans were found to be different than those for

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C. jejuni (Gillespie et al., 2002). This finding has emphasized the need for species-specific studies and for development of separate strategies for control of these two organisms (Tam, O'Brien, Adak, Meakins, & Frost, 2003).

Bacteria that invade the food chain may only be controlled by strategies that include the use of biocides as antiseptics and disinfectants (Beier et al., 2017). Chemicals that inhibit or kill a broad-spectrum of microorganisms are defined as disinfectants (White & McDermott, 2001). Food products that flow from the farm to the consumer's table must be guided by comprehensive strategies to control human pathogens (Wachsmuth, Sparling, Barrett, & Potter, 1997). Disinfectants are used in animal production, veterinary medicine, the food processing industry, human medicine, restaurants, and consumer's homes and often contain a variety of active ingredients in differing combinations (Beier et al., 2017). During application of disinfectants, the resultant concentrations used may be lower than required to kill the targeted bacteria (Chapman, 2003). These lower levels of disinfectants can result in the formation of biofilms and antimicrobial resistance (AMR) (Capita, Riesco-Paláez, Alonso-Hernando, & Alonso-Calleja, 2014). The emergence of multidrug-resistant pathogens worldwide as well as a lack of new drug development is a concern in both human and veterinary medicine (CDC, 2013; Ventola, 2015). Research studies show that there is bacterial resistance to biocides (Davin-Regli & Pagès, 2012; Maillard, 2007; Russell, 2002; Slipski, Zhanel, & Bay, 2018; Tumah, 2009), and biocide use has resulted in cross-resistance to antimicrobials (Al-Jailawi, Ameen, & Al-Jeboori, 2013; Beier, Bischoff, Ziprin, Poole, & Nisbet, 2005; Braoudaki & Hilton, 2004; Davin-Regli & Pagès, 2012; Gnanadhas, Marathe, & Chakravortty, 2013; Maris, 1991; Romaro, Burgos, Pérez-Pulido, Gálvez, & Lucas, 2017; Sidhu, Heir, Leegaard, Wiger, & Holck, 2002; Wales & Davies, 2015; Wand, Bock, Bonney, & Sutton, 2017) producing a real risk that increased biocide use may exacerbate the trend of increasing AMR in pathogenic organisms (Fraise, 2002).

Our laboratory has investigated the interactions of several different foodborne pathogens with disinfectants to better understand these important bacteria and have shown that didecyldimethylammmonium chloride (C10AC) was the most effective ammonium chloride against all pathogenic bacteria studied as well as in the commercial disinfectant P-128 (Beier et al., 2017). The current study characterizes the antimicrobial and disinfectant susceptibility profiles of 111 *C. coli* strains isolated in earlier studies evaluating pathogens in market age pigs (Harvey et al., 1999) and food animals and retail meat (NARMS, 2015). The objectives here were to evaluate the occurrence of AMR and disinfectant susceptibility in swine *C. coli* isolates obtained in 1998 to 1999 and in 2015. This work can be used to establish an understanding of the effects of 22 disinfectants and disinfectant components on *C. coli* and determine if cross-resistance between antibiotics and disinfectants is demonstrated and to compare the antimicrobial and disinfectant susceptibility in swine *C. coli* isolated during these two time periods.

Materials and Methods

Campylobacter coli strains

All *C. coli* strains evaluated here were previously isolated from cecal contents ($n = 7$), feces ($n = 5$), and rectal swabs ($n = 51$) of market age pigs (Harvey et al., 1999), and from the cecal contents of market age pigs ($n = 16$), sows ($n = 20$), and commercial pork chops ($n = 12$) (NARMS, 2015). All 111 *C. coli* strains were cultured in our laboratory for 48 hr at 42 °C on trypticase soy agar containing 5% sheep blood (BBL Stacker Plates; Becton, Dickinson and Company, Sparks, MD, USA) in a microaerophilic atmosphere of 10% CO_2, 5% O_2, and 85% N_2. The strains were transferred to a ferrous sulfate hydrate, sodium metabisulfite, and sodium pyruvate additive prepared medium (FBP medium) for cryopreservation (Beier et al., 2018; Gorman & Adley, 2004), and stored at –80 °C.

Susceptibility testing

Antimicrobial susceptibility testing. *C. coli* minimum inhibitory concentrations (MICs) were determined against antimicrobials using broth microdilution methods according to the Clinical and Laboratory Standards Inst. (CLSI) (CLSI, 2013; 2015), and the methods of TREK Diagnostic Systems for susceptibility using antimicrobial susceptibility testing (AST) *Campylobacter* Sensititre® plates (TREK, 2018). The AST *Campylobacter* Sensititre® plates (CAMPY) were used to evaluate the antimicrobial MICs, and Sensititre® cation-adjusted Mueller-Hinton broth (MHB) with TES (Tris, EDTA, and NaCl, pH 8) (5 mL tubes), Sensititre® cation-adjusted MHB w/TES w/Lysed horse blood (11 mL tubes), doseheads (#E3010), and the 0.5 McFarland standard were obtained from Remel (Lenexa, KS, USA). *C. coli* strains were incubated for 48 hr at 42 °C for broth microdilution testing because some strains did not sufficiently grow in 24 hr and methods were similar to previously described (Beier et al., 2018). *C. coli* MICs of the following nine antimicrobials, azithromycin (AZI), ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERY), florfenicol (FFN), gentamicin (GEN), nalidixic acid (NAL), telithromycin (TEL), and tetracycline (TET) (Table 1 to 3) were determined using the Sensititre® susceptibility system according to the instructions from Trek Diagnostic Systems (Thermo Fisher Scientific, Oakwood Village, OH, USA). *C. jejuni* ATCC 33560 was used as control for AST. *C. coli* MICs were determined to be the lowest concentration of the chemical that showed no visible growth of the organism (Andrews, 2001) as observed on a SensiTouch® imaging system (TREK Diagnostic Systems Ltd., East Grinsted, UK).

Disinfectant susceptibility testing. Twenty-two disinfectants and disinfectant components were tested against the *C. coli* strains in this study. The recommended uses and sources for these disinfectants were previously reported (Beier et al., 2017), the exponent "^{CP}" was added to their names to indicate a Commercial Product, and the abbreviations are listed as follows (name, abbreviation): benzalkonium chloride, BKC; Betadine first aid solution^{CP} (10% povidone-iodine), P-I; cetylpyridinium bromide hydrate, CPB; DC&R^{CP}, DC&R^{CP}; ethylhexadecyldimethylammonium bromide, CDEAB; Food Service Sanitizer^{CP}, FSS; F-25 Sanitizer^{CP}, F25; Final Step 512 Sanitizer^{CP}, FS512; hexadecylpyridinium chloride, CPC; hexadecyltrimethylammonium bromide, CTAB; Novasan Solution^{CP} (chlorhexidine diacetate), chlorhexidine; Odoban^{CP}, Odoban^{CP}; P-128^{CP}, P-128^{CP}; Tek-Trol^{CP}, Tek-Trol^{CP}; triclosan (ergasan), triclosan; didecyldimethylammonium chloride, C10AC; benzyldimethyldodecylammonium chloride, C12BAC; benzyldimethyltetradecylammonium chloride, C14BAC; benzyldimethylhexadecylammonium chloride, C16BAC; J.T. Baker 37% formaldehyde solution, formaldehyde; and tris(hydroxylmethyl)nitromethane, THN; and dioctyldimethylammonium chloride, C8AC, was obtained from Lonza Inc. (Fairlawn, NJ, USA). Dimethyl sulfoxide (DMSO) (MilliporeSigma, St. Louis, MO, USA) was used to aid in solubilizing some disinfectants. $^{RO}H_2O$ was produced by a reverse osmosis

Table 1–Antimicrobial resistance profiles among 63 *Campylobacter coli* strains isolated in 1998 to 1999 from swine cecal contents, rectal swabs, and feces.

Antimicrobial	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	Range of MICs (μg/mL)	No. (%) Resistant	Breakpoint
Aminoglycosides					
Gentamicin	0.5	0.5	0.25 to 0.5	0 (0)	≥8
Fluoroquinolones and quinolones					
Ciprofloxacin	0.06	0.12	0.03 to 0.12	0 (0)	≥1
Nalidixic acid	≤4	8	≤4 to 64	1 (1.6)	≥64
Ketolides					
Telithromycin	0.25	1	0.06 to 2	0 (0)	≥16
Lincomycins					
Clindamycin	0.12	0.5	0.06 to 2	0 (0)	≥8
Macrolides					
Azithromycin	0.03	0.06	≤0.015 to 0.25	0 (0)	≥8
Erythromycin	0.25	1	0.06 to 1	0 (0)	≥32
Phenicols					
Florfenicol	0.5	1	0.12 to 2	0 (0)	≥8
Tetracyclines					
Tetracycline	8	>64	0.12 to >64	28 (44.4)	≥16

system obtained from MilliporeSigma (Bedford, MA, USA). Some disinfectants exist as mixtures of multiple active components: The mixtures evaluated here, and their percentage of active ingredients, were previously listed (Beier et al., 2017). Since DC&R^{CP}, Tek-$Trol^{CP}$, FSS, F25, FS512, and P-128^{CP} are mixtures of several active disinfectant components, the *C. coli* MICs for these disinfectants were determined on the original mixtures. The susceptible/resistant criterion for bacteria used by Heath and Rock (2000) for triclosan was used; bacteria with MICs < 0.5 μg/mL were susceptible, and bacteria with MICs > 2 μg/mL were resistant to triclosan. The chlorhexidine breakpoint was used as defined by Leelaporn, Paulsen, Tennent, Littlejohn, and Skurray (1994) for staphylococci; bacteria with MICs ≥ 1 μg/mL were resistant. The resistance criterion for BKC defined by Sidhu, Sørum, and Holck (2002) for Gram-negative bacteria was used in this study; bacteria with MICs < 30 μg/mL were susceptible, low-level resistance was assigned to bacteria with MICs from 30 to 50 μg/mL, and bacteria with MICs > 50 μg/mL were resistant to BKC.

$^{RO}H_2O$ was used to make dilutions of the disinfectants and disinfectant components, and prior to use the solutions were filter sterilized using 0.2 μm × 25 mm syringe filters (No. 431224, Corning Inc., Corning, NY, USA). Some disinfectants required DMSO to be added to allow more concentrated solutions to be produced. DMSO was added to the following disinfectants and components: triclosan (% DMSO added = 80%, % DMSO in final solution = 4%), C14BAC (20%, 1%), C16BAC (60%, 3%), THN (60%, 5%), CPB (100%, 4%), and CTAB (100%, 4%). The amount of DMSO contained in the final working solutions did not exceed 5%. The method used for disinfectant susceptibility determination was similar to that used for disinfectant susceptibility testing (DST) of beta-hemolytic *Escherichia coli* (Beier et al., 2005) and *Salmonella* from cattle (Beier et al., 2017). The following concentrations of disinfectants were tested and the results are presented in Table 5 to 7: CPB, 0.125 to 128 μg/mL; CTAB, 0.125 to 128 μg/mL; C8AC, 0.0625 to 64 μg/mL; C16BAC, 0.0625 to 64 μg/mL; Tek-$Trol^{CP}$, 0.25 to 256 μg/mL; THN, 2 to 2048 μg/mL; triclosan, 0.0625 to 64 μg/mL; and the rest of the disinfectant concentrations used were equal to those previously published (Beier et al., 2017). The control organism used during microaerobic disinfectant testing was *C. jejuni* ATCC 33560. *E. coli* ATCC 25922 disinfectant susceptibility results obtained in aerobic conditions were also compared, since ATCC 25922 was previously used as the control organism during aerobic susceptibility testing (Beier et al., 2017).

Calculation of component MICs for disinfectants containing multiple components

The following calculations allow the determination of hypothetical MICs (^{hypo}MICs) for each component in a multiple-component disinfectant.

Calculation of the ^{hypo}MICs for all active components of DC&R^{CP}. The disinfectant DC&R^{CP} is a mixture of three active components, THN 19.2%, BACs 3.08% (mainly comprised of C12BAC, C14BAC, and C16BAC), and formaldehyde (Form) 2.28%. The ^{hypo}MICs of the individual active components of DC&R^{CP} can be calculated by multiplying the DC&R^{CP} MICs for the 1998 to 1999 isolates (Table 5) and for the 2015 isolates (Table 6) by the percentage of the component of interest, and then dividing the result by the sum of percentages for all active components in DC&R^{CP}, as previously described (Beier et al., 2017). The $^{1998–1999}$DC&R_{BAC} ^{hypo}MICs for the $^{1998–1999}$DC&R^{CP} MICs of ≤1, 2, 4, 8, 16, and 32 μg/mL (Table 5) were calculated. The ^{2015}DC&R_{BAC} ^{hypo}MICs for the ^{2015}DC&R^{CP} MICs of ≤1, 2, 4, 8, and 16 μg/mL (Table 6) were calculated. Similarly, the DC&R_{THN} ^{hypo}MICs and DC&R_{FORM} ^{hypo}MICs were calculated from the DC&R^{CP} MICs for both the 1998 to 1999 and 2015 isolates.

Calculation of the ^{hypo}MICs for all active components of P-128^{CP}. The disinfectant P-128^{CP} contains the active components C10AC 5.07% and the BACs 3.38%. The ^{hypo}MICs of the individual active components of P-128^{CP} were calculated in a similar way as the DC&R^{CP} active components above. Briefly, the $^{1998–1999}$P-128^{CP} MICs of 0.25, 0.5, 1, 2, 4, and 8 μg/mL (Table 5) were multiplied by the percentage of the active component of interest and divided by the sum of all active component percentages in P-128^{CP}. The multiplier for the BACs is 3.38/8.45 and the multiplier for C10AC is 5.07/8.45. The calculation of $^{1998–1999}$P-128^{CP} MICs results in the $^{1998–1999}$P-128_{BAC} ^{hypo}MICs and $^{1998–1999}$P-128_{C10AC} ^{hypo}MICs, and the calculation of the ^{2015}P-128^{CP} MICs of 0.5, 1, 2, 4, 8, and 16 (Table 6) with the two multipliers affords the ^{2015}P-128_{BAC} ^{hypo}MICs and the ^{2015}P-128_{C10AC} ^{hypo}MICs.

Table 2–Antimicrobial resistance profiles among 36 *Campylobacter coli* cecal strains isolated in 2015 from swine.

Antimicrobial	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	Range of MICs (μg/mL)	No. (%) Resistant	Breakpoint
Aminoglycosides					
Gentamicin	0.5	0.5	0.25 to 1	0 (0)	≥8
Fluoroquinolones and quinolones					
Ciprofloxacin	0.06	0.25	0.03 to 8	3 (8.3)	≥1
Nalidixic acid	≤4	8	≤4 to >64	1 (2.8)	≥64
Ketolides					
Telithromycin	1	>8	0.12 to >8	9 (25)	≥16
Lincomycins					
Clindamycin	0.25	8	0.06 to 16	10 (27.8)	≥8
Macrolides					
Azithromycin	0.06	>64	≤0.015 to >64	12 (33.3)	≥8
Erythromycin	1	>64	0.12 to >64	12 (33.3)	≥32
Phenicols					
Florfenicol	0.5	1	0.12 to 1	0 (0)	≥8
Tetracyclines					
Tetracycline	64	>64	0.12 to >64	27 (75)	≥16

Table 3–Antimicrobial resistance profiles among 12 *Campylobacter coli* strains isolated in 2015 from commercial pork chops.

Antimicrobial	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	Range of MICs (μg/mL)	No. (%) Resistant	Breakpoint
Aminoglycosides					
Gentamicin	0.5	0.5	0.25 to 1	0 (0)	≥8
Fluoroquinolones and quinolones					
Ciprofloxacin	0.06	0.12	0.03 to 4	1 (8.3)	≥1
Nalidixic acid	≤4	8	≤4 to 64	1 (8.3)	≥64
Ketolides					
Telithromycin	1	8	0.12 to >8	2 (16.7)	≥16
Lincomycins					
Clindamycin	0.5	8	0.12 to 16	4 (33.3)	≥8
Macrolides					
Azithromycin	0.06	>64	0.03 to >64	5 (41.7)	≥8
Erythromycin	1	>64	0.12 to >64	5 (41.7)	≥32
Phenicols					
Florfenicol	0.5	1	0.25 to 2	0 (0)	≥8
Tetracyclines					
Tetracycline	64	>64	1 to >64	9 (75)	≥16

Results and Discussion

Antimicrobial resistance

The AMR profiles of 63 *C. coli* strains isolated in 1998 to 1999 obtained from market age swine cecal contents, rectal swabs, and feces (Table 1) and the AMR profiles of 36 *C. coli* strains isolated in 2015 from swine cecal contents (Table 2) are defined by the MIC_{50} and MIC_{90} (the lowest concentration of antimicrobial at which 50% and 90% of the isolates are inhibited, respectively), the range of MICs, the number and percent of organisms resistant, and the breakpoints for the antimicrobials tested. The 1998 to 1999 strains demonstrated resistance only to nalidixic acid (1.6%) and TET (44.4%) of the antimicrobials tested. However, the 2015 swine strains showed no resistance to only gentamicin and florfenicol. The 2015 strains showed 25% or greater resistance to four antibiotics, telithromycin, clindamycin, azithromycin, and erythromycin, while the strains demonstrated 75% resistance to TET. The 2015 strains showed much more resistance to antibiotics than the 1998 to 1999 strains. Table 3 displays the AMR profiles among 12 *C. coli* strains obtained during 2015 from commercial pork chops. The AMR results for the commercial pork chop strains are quite similar to the AMR results obtained from the other swine strains isolated during 2015, but the pork chop strains show higher resistance to clindamycin and the macrolides, while the resistance to TET remains the same (75%). These data demonstrate higher AMR in *C. coli* strains isolated during 2015 than strains isolated earlier during 1998 to 1999. See Table S1 for AMR among 7 *C. coli* cecal strains from market age swine (1998 to 1999), Table S2 for AMR among 5 *C. coli* fecal strains from market age swine (1999), Table S3 for AMR among 51 *C. coli* rectal swab strains from market age swine (1998 to 1999), Table S4 for AMR among 16 *C. coli* cecal strains from market age swine (2015), and Table S5 for AMR among 20 *C. coli* cecal strains from sows (2015).

Campylobacter coli AMR profiles

The percentage of AMR among the 111 *C. coli* strains tested is presented in Table 4, which shows the total number of strains from each source, the number and percentage of AMR strains, and the number of the most common resistance profiles. There were a total of 63 *C. coli* cecal strains from 1998 to 1999 market age swine tested and the common resistance profile among these strains was TET, with one strain having resistance to NAL. There were also 48 *C. coli* strains isolated in 2015 and the most common AMR profile was TET, followed by AMR profiles of AZI-ERY-TET-TEL-CLI, AZI-ERY-TET-CLI, and CIP-TET-NAL. There were higher

Table 4–The antimicrobial resistance and resistance profiles among 111 *Campylobacter coli* strains isolated in 1998 to 1999 and 2015 from swine.

Year (source)	Total number of *C. coli* strains	Number of strains resistant to antimicrobials[a] (%)	Common resistance profiles (number of strains – profile)
1998 to **1999**			
Cecal strains from market age swine	7	3 (43)	3 – TET
Fecal strains from market age swine	5	3 (60.0)	2 – TET 1 – NAL
Rectal swab strains from market age swine	51	23 (45.1)	23 – TET
2015			
Cecal strains from market age swine	16	14 (87.5)	6 – TET 2 – AZI-ERY-TET-TEL-CLI 2 – AZI-ERY-TET-CLI 2 – CIP-TET-NAL 1 – AZI-ERY 1 – CIP-NAL
Cecal strains from sows	20	15 (75)	8 – TET 6 – AZI-ERY-TET-TEL-CLI 1 – AZI-ERY-TET-CLI
Strains from commercial pork chops	12	10 (83.3)	4 – TET 2 – AZI-ERY-TET-TEL-CLI 1 – AZI-ERY-TET-CLI 1 – AZI-ERY-CLI 1 – AZI-ERY-TET 1 – CIP-TET-NAL
Overall total	111	68 (61.3)	

[a]Antimicrobials evaluated were the following: Aminoglycosides: GEN, gentamicin; Fluoroquinolones and Quinolones: CIP, ciprofloxacin; NAL, nalidixic acid; Ketolides: TEL, telithromycin; Lincomycins: CLI, clindamycin; Macrolides: AZI, azithromycin; ERY, erythromycin; Phenicols: FFN, florfenicol; and Tetracyclines: TET, tetracycline.

percentages of strains resistant to antibiotics in the 2015 strains than in the 1998 to 1999 strains. The AMR profiles of the commercial pork chop strains were similar to the resistance profiles of the 2015 cecal strains from market age swine.

Disinfectant susceptibility

Table 5 and 6 show the results of the DST with the distribution of disinfectant and disinfectant component susceptibility profiles of 63 and 36 *C. coli* strains from swine isolated in 1998 to 1999 and 2015, respectively. All 99 *C. coli* swine strains were resistant to triclosan. However, the triclosan MIC_{50} for the 2015 strains (Table 6) was higher than the MIC_{50} of the 1998 to 1999 strains (Table 5). Previously, *Salmonella* strains from turkeys (Beier et al., 2011) and cattle (Beier et al., 2017), *E. coli* O157:H7 strains (Beier et al., 2013), and non-O157 STEC strains (Beier et al., 2016) were all susceptible at ≤1μg/mL triclosan. But VRE strains (Beier et al., 2008) and *Pseudomonas aeruginosa* strains (Beier et al., 2014) were resistant to triclosan, as were all the *C. coli* strains evaluated here. Triclosan has long been described as a biocide since it is a synthetic product, but triclosan functions like an antibiotic as it has a specific cellular target (Webber et al., 2017). In *E. coli*, triclosan inhibits the highly conserved enzyme enoyl-acyl carrier protein reductase (ENP), which is the last enzyme in the bacterial fatty-acid biosynthesis elongation cycle (Heath & Rock, 2000). Recently, triclosan has been shown to induce multidrug resistance in *E. coli* through genetic mutations of at least five different genes, and upregulates genes encoding for beta-lactamases and efflux pumps, and downregulates genes relating to membrane permeability (Lu et al., 2018). In this study, we refer to triclosan as a pseudo-antibiotic since it is a synthetic product but has similar properties to an antibiotic and functions like an antibiotic.

Ninety-two percent of the *C. coli* strains (58/63) isolated from 1998 to 1999 swine samples demonstrated chlorhexidine resistance. The *C. coli* strains (36/36) isolated in 2015 (Table 6) were all resistant to chlorhexidine and the MIC_{50} and MIC_{90} of the 2015 strains also were higher than the 1998 to 1999 strains. Previously, VRE (Beier et al., 2008) and non-O157 STEC (Beier et al., 2016) strains were susceptible to chlorhexidine demonstrating MICs of ≤1μg/mL, whereas a prevalence of 4.3% of the *E. coli* O157:H7 strains was resistant to chlorhexidine (Beier et al., 2013), but *Salmonella* from turkeys (Beier et al., 2011), cattle (Beier et al., 2017), and *P. aeruginosa* (Beier et al., 2014) strains were resistant to chlorhexidine demonstrating similar MICs as those of the *C. coli* strains in this study.

All 99 *C. coli* swine strains were susceptible to BKC. But both the BKC MIC_{50} and MIC_{90} values for the 2015 strains (Table 6) were higher than the MIC_{50} and MIC_{90} values for the 1998 to 1999 strains (Table 5). Previously, VRE strains from community waste water (Beier et al., 2008) were also shown to be susceptible to BKC; however, 98.5% of the *Salmonella* strains from turkeys (Beier et al., 2011), 3.5% of the *E. coli* O157:H7 strains from cattle (Beier et al., 2013), 1.5% of non-O157 STEC strains from food animals and humans (Beier et al., 2016), and 27.6% of the *Salmonella* strains from cattle (Beier et al., 2017) demonstrated low level resistance to BKC. However, all 175 *P. aeruginosa* strains tested (Beier et al., 2014) were highly resistant to BKC. The disinfectants Tek-Trol^{CP} and P-I, and the disinfectant components, THN and formaldehyde, have high susceptibilities, and the highest measured susceptibilities were for P-I at 512 to 4096 μg/mL. An application rate of 100,000 μg/mL of P-I is recommended to be directly applied on wound surfaces. Therefore, the recommended application rate would be about a 24- to 195-fold excess over the required amount needed for disinfection of the *C. coli* tested here. The manufacturers of DC&R^{CP} and Tek-Trol^{CP} recommend application rates of 1919 and 1016 μg/mL for these disinfectants, respectively, and the observed *C. coli* MICs were below these application rates. However, the observed *C. coli* MICs for Tek-Trol^{CP} were within fourfold of the application rate, and therefore, care should be taken when making the application dilutions of this disinfectant. Also, excess liquid in the areas of application of diluted Tek-Trol^{CP} may

Table 5–Distribution of disinfectant and disinfectant component susceptibility profiles of 63 *Campylobacter coli* strains isolated in 1998 to 1999 from swine cecal contents, rectal swabs, and feces.

	MIC (µg/mL)																			MIC_{50}	MIC_{90}
Disinfectant[a]	**≤0.12**	**0.12**	**≤0.25**	**0.25**	**0.5**	**≤1**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**>64**	**128**	**256**	**512**	**1024**	**2048**	**4096**	**(µg/mL)**	**(µg/mL)**
DC&R[CP]						4[b]		16	26	11	5	1									4	8
Tek-Trol[CP]												2	45		16						64	128
Chlorhexidine[c]		2		3	5		25[d]	24	4												1[d]	2
Triclosan[e]											9[d]	30	23	1							32[d]	64
P-128[CP]				1	8		26	17	10	1											1	4
BKC			7		6		27	16	6	6	1										1	4
P-I																		5	57	1	2048	2048
FSS				5	13		25	18	2												1	2
F25	1			5	12		30	12	3												1	2
FS512				8	20		20	13	2												1	2
OdoBan[CP]			8		12		24	15	3		1										1	2
CPB					3		8	24	21	7											2	8
CPC			1		2		8	24	22	6											2	4
CDEAB					1		1	23	28	10											4	8
CTAB							2	3	38	20											4	8
C8AC[f]				2	5		16	19	19	2											2	4
C10AC[f]					5		26	24	6	2											2	4
C12BAC[f]			10		8		15	14	8	5	3										1	8
C14BAC[f]			1	1	4		19	22	12	4											2	4
C16BAC[f]								1	17	35	10										8	16
THN[f]										1	11	29	20		1	1					32	64
Formaldehyde[f]										1	41	18	2			1					16	32

[a]Disinfectant and disinfectant component abbreviations: BKC, benzalkonium chloride; chlorhexidine, Novasan Solution[CP]; CPB, cetylpyridinium bromide hydrate; CPC, hexadecylpyridinium chloride monohydrate; CDEAB, ethylhexadecyldimethylammonium bromide; CTAB, hexadecyltrimethylammonium bromide; FS512, Final Step 512 Sanitizer[CP]; FSS, Food Service Sanitizer[CP]; F25, F-25 Sanitizer[CP]; P-I, providone-iodine[CP]; C8AC, dioctyldimethylammonium chloride; C10AC, didecyldimethylammonium chloride; C12BAC, benzyldimethyldodecylammonium chloride; C14BAC, benzyldimethyltetradecylammonium chloride; C16BAC, benzyldimethylhexadecylammonium chloride; THN, tris(hydroxylmethyl)nitromethane; and [CP] = commercial product.

[b]Number of strains at this MIC.

[c]MICs ≥1 µg/mL are considered resistant for chlorhexidine (Leelaporn et al., 1994).

[d]The entries in RED indicate resistance.

[e]MICs >2 µg/mL are considered resistant for triclosan (Heath & Rock, 2000).

[f]This entry is a disinfectant component.

Table 6–Distribution of disinfectant and disinfectant component susceptibility profiles of 36 *Campylobacter coli* cecal strains isolated in 2015 from swine.

	MIC (µg/mL)																			MIC_{50}	MIC_{90}
Disinfectant[a]	**≤0.12**	**0.12**	**≤0.25**	**0.25**	**0.5**	**≤1**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**>64**	**128**	**256**	**512**	**1024**	**2048**	**4096**	**(µg/mL)**	**(µg/mL)**
DC&R[CP]						2[b]		5	17	10	2										4	8
Tek-Trol[CP]											1		24		9	2					64	128
Chlorhexidine[c]					1		9[d]	22	3	1											2[d]	4
Triclosan[e]											4[d]	12	18	2							64[d]	64
P-128[CP]					4		17	7	1	7											1	8
BKC			1		2		6	12	11	3	1										2	8
P-I																	2	3	31		2048	2048
FSS					5		11	16	3	1											2	4
F25				1	3		13	16	2	1											2	2
FS512				1	4		17	13	1												1	2
OdoBan[CP]					6		10	10	6	3	1										2	8
CPB					1		4	7	19	4	1										4	8
CPC					2		2	5	21	4	2										4	8
CDEAB							3	7	19	6	1										4	8
CTAB							1	4	18	13											4	8
C8AC[f]							5	9	15	6	1										4	8
C10AC[f]					6		13	13	2	2											1	4
C12BAC[f]			1		3		4	16	10	2											2	4
C14BAC[f]					1		5	22	8												2	4
C16BAC[f]									7	20	9										8	16
THN[f]										1	6	19	10								32	64
Formaldehyde[f]										2	27	7									16	32

[a]Disinfectant and disinfectant component abbreviations: BKC, benzalkonium chloride; chlorhexidine, Novasan Solution[CP]; CPB, cetylpyridinium bromide hydrate; CPC, hexadecylpyridinium chloride monohydrate; CDEAB, ethylhexadecyldimethylammonium bromide; CTAB, hexadecyltrimethylammonium bromide; FS512, Final Step 512 Sanitizer[CP]; FSS, Food Service Sanitizer[CP]; F25, F-25 Sanitizer[CP]; P-I, providone-iodine[CP]; C8AC, dioctyldimethylammonium chloride; C10AC, didecyldimethylammonium chloride; C12BAC, benzyldimethyldodecylammonium chloride; C14BAC, benzyldimethyltetradecylammonium chloride; C16BAC, benzyldimethylhexadecylammonium chloride; THN, tris(hydroxylmethyl)nitromethane; and [CP] = commercial product.

[b]Number of strains at this MIC.

[c]MICs ≥1 µg/mL are considered resistant for chlorhexidine (Leelaporn et al., 1994).

[d]The entries in RED indicate resistance.

[e]MICs >2 µg/mL are considered resistant for triclosan (Heath & Rock, 2000).

[f]This entry is a disinfectant component.

cause this disinfectant to not be effective. The disinfectants FSS, F25, FS512, OdoBan^{CP}, CPB, CPC, CDEAB, and CTAB have similar susceptibilities and have no defined breakpoints. But here again, both the MIC_{50} and MIC_{90} values for the 2015 strains (Table 6) against the disinfectants FSS, OdoBan^{CP}, and CPC were higher than the MIC_{50} and MIC_{90} values for the 1998 to 1999 strains (Table 5). Also, the triclosan, F25, and CPB MIC_{50} values for the 2015 strains (Table 6) were higher than the MIC_{50} values for the 1998 to 1999 strains (Table 5), resulting in 60% of disinfectants showing a trend toward higher susceptibility in the 2015 *C. coli* isolates. Therefore, the disinfectants and the antibiotics show a trend in *C. coli* isolated in 2015 compared with those from 1998 to 1999 to higher susceptibility or resistance.

Table 7 shows the distribution of disinfectant and disinfectant component susceptibility profiles for the 12 *C. coli* strains isolated in 2015 from commercial pork chops. For many disinfectants in Table 7, there is no remarkable change from the results for the 36 unprocessed swine strains shown in Table 6. However, in the case of BKC, FSS, and F25, both the MIC_{50} and MIC_{90} values are higher for the 2015 swine strains (Table 6) than those for the 2015 pork chop strains (Table 7). The MIC_{50} and MIC_{90} values for chlorhexidine and CPC are higher for the 2015 pork chop strains than the 1998 to 1999 swine strains (Table 5). The MIC_{50} values for triclosan and CPB are higher, as well as the MIC_{90} values of P-128 and OdoBan for the 2015 pork chop strains (Table 7) than those for the 1998 to 1999 strains (Table 5). Suggesting that the 2015 pork chop strains (Table 7) are similar to the 2015 cecal strains (Table 6), and both groups show higher susceptibilities for some disinfectants than the strains isolated in 1998 to 1999 (Table 5) from swine cecal contents, rectal swabs, and feces. For more detailed information, see Table S6 to S10 for disinfectant and disinfectant component susceptibility profiles of 7 *C. coli* cecal strains isolated in 1998 to 1999 from market age swine, for 5 *C. coli* fecal strains isolated in 1999 from market age swine, for 51 *C. coli* rectal swab strains isolated in 1998 to 1999 from market age swine, for 16 *C. coli* cecal strains isolated in 2015 from market age swine, and for 20 *C. coli* sow cecal strains isolated in 2015, respectively.

Comparison of *C. coli* inhibition by ammonium chloride disinfectant components

Figure 1 shows the number of strains at the molar MICs (MIC_Ms) for the ammonium chloride disinfectant components against the 111 *C. coli* strains in this study. Since the MIC_{50} and MIC_{90} values for C10AC, C12BAC, C14BAC, and C16BAC were equivalent for 1998 to 1999 strains and 2015 strains, ammonium chloride disinfectant components from both times were treated together in Figure 1. Based on these data, C10AC and C14BAC appear to be the most effective at inhibiting *C. coli*, while C8AC and C12BAC have intermediate activity and C16BAC was not efficient at inhibiting the *C. coli* strains tested here. There appears to be little difference in inhibition by C10AC and C14BAC in these *C. coli* strains. Previous studies demonstrated that C10AC was more effective than the other ammonium chloride components at inhibiting VRE strains (Beier et al., 2008), *E. coli* O157:H7 strains (Beier et al., 2013), *P. aeruginosa* strains (Beier et al., 2014), and non-O157 STEC strains (Beier et al., 2016), and was also more effective at inhibiting *Salmonella* derived from turkeys (Beier et al., 2011) and cattle (Beier et al., 2017). To view interaction details of ammonium chloride disinfectant components with the various *C. coli* strains isolated at the two time periods of 1998 to 1999 and 2015, see Figure S1 to S6 for concentrations of ammonium chloride disinfectant components at the MIC_Ms of the 7 *C. coli*

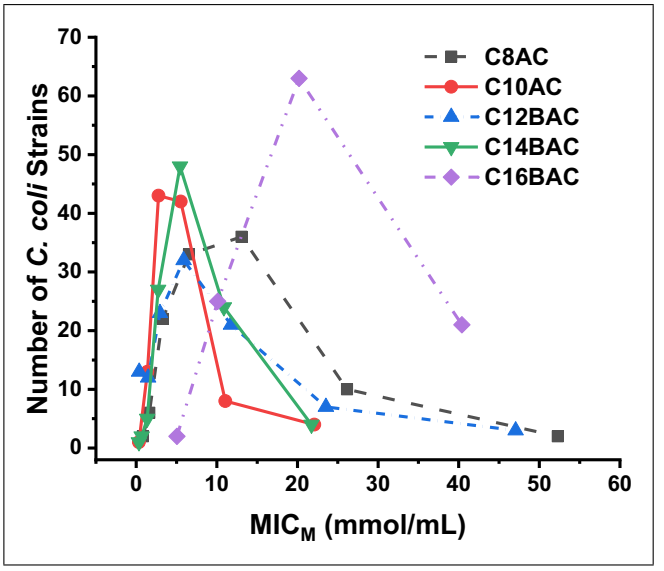

Figure 1—Concentrations of the ammonium chloride disinfectant components at the molar MICs (MIC_Ms) against 111 *Campylobacter coli*.

cecal strains isolated in 1998 to 1999 from market age swine, 5 *C. coli* fecal strains isolated in 1999 from market age swine, 51 *C. coli* rectal swab strains isolated in 1998 to 1999 from market age swine, 16 *C. coli* cecal strains isolated in 2015 from market age swine, 20 *C. coli* cecal strains isolated in 2015 from sows, and 12 *C. coli* strains isolated in 2015 from commercial pork chops, respectively. Also, see Table S11 to S15 for the susceptibility values obtained for C8AC, C10AC, C12BAC, C14BAC, and C16BAC, respectively, among the 63 *C. coli* swine strains isolated in 1998 to 1999 and the 48 *C. coli* swine and pork chop strains isolated in 2015.

Calculated ^{hypo}MICs for disinfectants containing multiple components

The ^{hypo}MICs can be compared to the actual MICs of the individually tested components thereby determining which component of the multiple-component disinfectant is responsible for the disinfectant activity against *C. coli*. The $^{1998–1999}$DC&R_{BAC} ^{hypo}MICs for the $^{1998–1999}$DC&R^{CP} MICs (Table 5) were calculated to be $^{1998–1999}$DC&R_{BAC} ^{hypo}MICs of ≤0.125, 0.25, 0.5, 1, 2, and 4 μg/mL, and for the ^{2015}DC&R^{CP} MICs (Table 6) were calculated to be ^{2015}DC&R_{BAC} ^{hypo}MICs of ≤0.125, 0.25, 0.5, 1, and 2 μg/mL. The calculated values for the DC&R_{BAC} ^{hypo}MICs are nearly identical to the MIC values shown in Table 5 and 6 for the individual C12BAC and C14BAC components. The higher ^{hypo}MICs are also the same as observed for the individual C16BAC component MICs (Table 5 and 6). Therefore, the BAC component of DC&R^{CP} fits the MIC pattern required for DC&R^{CP} inhibition of *C. coli*. Similarly, the DC&R^{CP} MICs were calculated to give the $^{1998–1999}$DC&R_{THN} ^{hypo}MICs of ≤0.78, 1.56, 3.13, 6.25, 12.51, and 25.02 μg/mL and ^{2015}DC&R_{THN} ^{hypo}MICs to be ≤0.78, 1.56, 3.13, 6.25, and 12.51 μg/mL. For the most part, these DC&R_{THN} ^{hypo}MICs are too low for THN to inhibit *C. coli*. Also, the DC&R^{CP} MICs were calculated to give the $^{1998–1999}$DC&R_{FORM} ^{hypo}MICs of ≤0.093, 0.017, 0.371, 0.743, 1.49, and 2.97 μg/mL, and ^{2015}DC&R_{FORM} ^{hypo}MICs of ≤0.093, 0.017, 0.371, 0.743, and 1.49 μg/mL. Compared with the authentic formaldehyde MICs, the DC&R_{FORM} ^{hypo}MICs are also too low for formaldehyde to inhibit *C. coli*. Therefore, the BAC components of DC&R^{CP}, specifically C12BAC and C14BAC, are the

Table 7–Distribution of disinfectant and disinfectant component susceptibility profiles of 12 *Campylobacter coli* strains isolated in 2015 from commercial pork chops.

	MIC (µg/mL)																			MIC_{50}	MIC_{90}
Disinfectant[a]	**≤0.12**	**0.12**	**≤0.25**	**0.25**	**0.5**	**≤1**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**>64**	**128**	**256**	**512**	**1024**	**2048**	**4096**	**(µg/mL)**	**(µg/mL)**
DC&R[CP]								3[b]	4	3	1	1									4	16
Tek-Trol[CP]													6		5	1					64	128
Chlorhexidine[c]				1			3[d]	5	2	1											2[d]	4
Triclosan[e]												5[d]	6	1							64[d]	64
P-128[CP]					3		3	4		1	1										1	8
BKC					3		3	2	3	1											1	4
P-I																			12		2048	2048
FSS				2			5	5													1	2
F25					3		5	4													1	2
FS512				2	1		5	3	1												1	2
OdoBan[CP]			1		2		4	3	1		1										1	4
CPB							1	1	7	3											4	8
CPC							1	4	2	5											4	8
CDEAB								1	10	1											4	4
CTAB									6	6											4	8
C8AC[f]					1		1	5	2	2	1										2	8
C10AC[f]		1			2		4	5													1	2
C12BAC[f]			2		1		4	2	3												1	4
C14BAC[f]				1			3	4	4												2	4
C16BAC[f]								1	1	8	2										8	16
THN[f]												6	6								32	64
Formaldehyde[f]											9	3									16	32

[a]Disinfectant and disinfectant component abbreviations: BKC, benzalkonium chloride; chlorhexidine, Novasan Solution[CP]; CPB, cetylpyridinium bromide hydrate; CPC, hexadecylpyridinium chloride monohydrate; CDEAB, ethylhexadecyldimethylammonium bromide; CTAB, hexadecyltrimethylammonium bromide; FS512, Final Step 512 Sanitizer[CP]; FSS, Food Service Sanitizer[CP]; F25, F-25 Sanitizer[CP]; P-I, providone-iodine[CP]; C8AC, dioctyldimethylammonium chloride; C10AC, didecyldimethylammonium chloride; C12BAC, benzyldimethyldodecylammonium chloride; C14BAC, benzyldimethyltetradecylammonium chloride; C16BAC, benzyldimethylhexadecylammonium chloride; THN, tris(hydroxylmethyl)nitromethane; [CP] = commercial product.

[b]Number of strains at this MIC.

[c]MICs ≥1 µg/mL are considered resistant for chlorhexidine (Leelaporn et al., 1994).

[d]The entries in RED indicate resistance.

[e]MICs >2 µg/mL are considered resistant for triclosan (Heath & Rock, 2000).

[f]This entry is a disinfectant component.

active components in DC&R^{CP} causing inhibition of *C. coli*. The result obtained here for *C. coli* is like previous reports describing the BAC component of DC&R^{CP} as the active component against Gram-positive VRE (Beier et al., 2008) and other Gram-negative bacteria (Beier et al., 2011; 2013; 2014; 2016; 2017). Therefore, these data demonstrate that THN and formaldehyde in DC&R^{CP} are not required for inhibition of *C. coli*, or VRE (Beier et al., 2008), *Salmonella* from turkeys (Beier et al., 2011), *E. coli* O157:H7 (Beier et al., 2013), *P. aeruginosa* (Beier et al., 2014), non-O157 STECs (Beier et al., 2016), or *Salmonella* from cattle (Beier et al., 2017). The addition of THN and formaldehyde to DC&R^{CP} results in only increasing the level of unnecessary chemicals introduced into the environment.

The calculation resulted in $^{1998–1999}$P-128$_{BAC}$ ^{hypo}MICs of 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 μg/mL, and in ^{2015}P-128$_{BAC}$ ^{hypo}MICs of 0.2, 0.4, 0.8, 1.6, and 3.2 μg/mL. Since the P-128$_{BAC}$ ^{hypo}MICs are generally high enough to cause the inhibition of *C. coli*, these BAC components can be considered the active components in P-128^{CP} against *C. coli*. This was not the case in previous studies of P-128^{CP} against VRE (Beier et al., 2008) and other Gram-negative pathogenic bacteria (Beier et al., 2011; 2013; 2014; 2016; 2017). In these previous studies, the BAC component of P-128^{CP} was not the major active component, but C10AC was the major active component in all cases. In a similar manner, the $^{1998–1999}$P-128$_{C10AC}$ ^{hypo}MICs were calculated to be 0.15, 0.3, 0.6, 1.2, 2.4, and 4.8 μg/mL, and the ^{2015}P-128$_{C10AC}$ ^{hypo}MICs were calculated to be 0.3, 0.6, 1.2, 2.4, and 4.8 μg/mL. Upon comparison with the individual $^{1998–1999}$C10AC MICs and ^{2015}C10AC MICs in Table 5 and 6, it is clear the lower P-128$_{C10AC}$ ^{hypo}MICs were not sufficient to inhibit *C. coli*. Those *C. coli* bacteria that required higher MICs may be inhibited by a synergistic effect from all three components, C10AC, C12BAC, and C14BAC.

Conclusions

TET resistance was observed in 44.4% of the 63 swine *C. coli* strains isolated in 1998 to 1999, and in 75% of both the 36 *C. coli* swine strains and the 12 commercial pork chop strains isolated in 2015. All other antibiotics tested resulted in a low or zero prevalence of AMR in the 63 1998 to 1999 *C. coli* swine strains. All 1998 to 1999 *C. coli* swine strains were highly susceptible to gentamicin, ciprofloxacin, nalidixic acid, telithromycin, clindamycin, azithromycin, erythromycin, and florfenicol. While both the 2015 *C. coli* swine strains and pork chop strains showed a marked increase in AMR to ciprofloxacin, telithromycin, clindamycin, azithromycin, and erythromycin. The primary overall antibiotic resistance profile observed in the 1998 to 1999 strains was TET. But the resistance profiles observed in the 2015 strains were TET followed by AZI-ERY-TET-TEL-CLI, AZI-ERY-TET-CLI, CIP-TET-NAL, and AZI-ERY (CLI or TET). All 111 strains were resistant to the disinfectant triclosan (a pseudo-antibiotic), and 84.1% of the 1998 to 1999 strains and 95.8% of the 2015 strains were resistant to chlorhexidine. All strains were susceptible to benzalkonium chloride. The disinfectants Tek-Trol^{CP} and providone-iodine^{CP}, and the disinfectant components, tris(hydroxylmethyl)nitromethane and formaldehyde, demonstrated the highest susceptibilities. There was a shift toward higher susceptibility to chlorhexidine, triclosan, P-128^{CP}, OdoBan^{CP}, CPB, and CPC in the *C. coli* strains obtained from commercial pork chops in 2015 and also to FSS and F25 in 2015 cecal strains compared with the 63 *C. coli* strains isolated in 1998 to 1999. Little difference was observed in the disinfectant components C10AC and C14BAC inhibition of *C. coli* strains, which was a different result from earlier observations with other pathogenic bacteria. The disinfectant ammonium chloride components C8AC and C12BAC showed intermediate *C. coli* inhibition, while C16BAC was not effective at inhibiting *C. coli* strains. By calculating the hypothetical MICs of the active components in the disinfectant DC&R^{CP} and comparing the results with the individual component MICs, it was determined that the ammonium chloride BAC components, C12BAC and C14BAC, were responsible for the observed inhibition of *C. coli* by the disinfectant DC&R^{CP}. In like manner, the calculated hypothetical MICs of the active components of P-128^{CP} showed that the same two BAC components, C12BAC and C14BAC, or a synergistic effect between C10AC, C12BAC, and C14BAC, were responsible for inhibition of *C. coli* strains by the disinfectant P-128^{CP}. The use of THN and formaldehyde in DC&R^{CP} is questionable because these components are not effective against *C. coli* at their included concentrations, nor have they been effective in previous studies with five other pathogenic bacteria species, and their inclusion only results in additional unwanted chemicals in the environment.

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Authors' Contributions

R. Beier conceptualized the work, conducted experiments, analyzed data, interpreted results, and drafted the manuscript. R. Harvey helped with project administration and edited the manuscript. C. Hernandez took part in the investigation, validation, and edited the manuscript. M. Hume edited the manuscript. K. Andrews took part in the investigation, validation, and edited the manuscript. R. Droleskey took part in the investigation, validation, and edited the manuscript. M. Davidson took part in project administration and edited the manuscript. S. Bodeis-Jones took part in the investigation and edited the manuscript. S. Young took part in the investigation and edited the manuscript. R. Anderson took part in project administration, supervision, and edited the manuscript. D. Nisbet took part in project administration, provided resources, and supervision.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Concentrations of the ammonium chloride disinfectant components at the molar MICs of seven *Campylobacter coli* cecal strains isolated in 1998 to 1999 from market age swine.

Figure S2. Concentrations of the ammonium chloride disinfectant components at the molar MICs of five *Campylobacter coli* fecal strains isolated in 1999 from market age swine.

Figure S3. Concentrations of the ammonium chloride disinfectant components at the molar MICs of 51 *Campylobacter coli* rectal swab strains isolated in 1998 to 1999 from market age swine.

Figure S4. Concentrations of the ammonium chloride disinfectant components at the molar MICs of 16 *Campylobacter coli* cecal strains isolated in 2015 from market age swine.

Figure S5. Concentrations of the ammonium chloride disinfectant components at the molar MICs of 20 *Campylobacter coli* cecal strains isolated in 2015 from sows.

Figure S6. Concentrations of the ammonium chloride disinfectant components at the molar MICs of 12 *Campylobacter coli* strains isolated in 2015 from commercial pork chops.

Table S1. Antimicrobial resistance profiles among seven *Campylobacter coli* cecal strains isolated in 1998 to 1999 from market age swine.

Table S2. Antimicrobial resistance profiles among five *Campylobacter coli* fecal strains isolated in 1999 from market age swine.

Table S3. Antimicrobial resistance profiles among 51 *Campylobacter coli* strains isolated in 1998 to 1999 from rectal swabs of market age swine.

Table S4. Antimicrobial resistance profiles among 16 *Campylobacter coli* cecal strains isolated in 2015 from market age swine.

Table S5. Antimicrobial resistance profiles among 20 *Campylobacter coli* cecal strains isolated in 2015 from sows.

Table S6. Distribution of disinfectant and disinfectant component susceptibility profiles of seven *Campylobacter coli* cecal strains isolated in 1998 to 1999 from market age swine.

Table S7. Distribution of disinfectant and disinfectant component susceptibility profiles of five *Campylobacter coli* isolated in 1998–1999 from market age swine fecal strains.

Table S8. Distribution of disinfectant and disinfectant component susceptibility profiles of 51 *Campylobacter coli* rectal swab strains isolated in 1998 to 1999 from market age swine.

Table S9. Distribution of disinfectant and disinfectant component susceptibility profiles of 16 *Campylobacter coli* cecal strains isolated in 2015 from market age swine.

Table S10. Distribution of disinfectant and disinfectant component susceptibility profiles of 20 *Campylobacter coli* isolated in 2015 from sow cecal strains.

Table S11. C8AC[A] susceptibility among the 63 *Campylobacter coli* strains isolated in 1998 to 1999 and the 48 *C. coli* strains isolated in 2015 from swine and pork chops.

Table S12. C10AC[A] susceptibility among the 63 *Campylobacter coli* strains isolated in 1998 to 1999 and the 48 *C. coli* strains isolated in 2015 from swine and pork chops.

Table S13. C12BAC[A] susceptibility among the 63 *Campylobacter coli* strains isolated in 1998 to 1999 and the 48 *C. coli* strains isolated in 2015 from swine and pork chops.

Table S14. C14BAC[A] susceptibility among the 63 *Campylobacter coli* strains isolated in 1998 to 1999 and the 48 *C. coli* strains isolated in 2015 from swine and pork chops.

Table S15. C16BAC[A] susceptibility among the 63 *Campylobacter coli* strains isolated in 1998 to 1999 and the 48 *C. coli* strains isolated in 2015 from swine and pork chops.